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Novartis AG
Lichtstrasse 35
4056 Basel
SUISSE

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Process for production of ionically crosslinked polysaccharide microspheres

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Process for production of ionically crosslinked polysaccharide microspheres

The present invention relates to a process for the production of microspheres comprising an ionically crosslinked polysaccharide and to a system for carrying out the process.

Polysaccharides which are copolymers of monosaccharides comprising a carboxylic acid group, herein referred to as "anionic polysaccharides", have found widespread application in formulation techniques. A particularly useful characteristic of many anionic polysaccharides is their ability to be readily soluble as free acids and/or salts of monovalent cations while forming strong gels on contact with divalent or polyvalent cations. Within the present application, anionic polysaccharides which instantaneously form gels by reaction with divalent or polyvalent cations, are called "ionically crosslinkable anionic polysaccharides".

Ionically crosslinkable anionic polysaccharides are, for example, alginic acid, some forms of carrageenan, Gellan gum, pectins, and cellulose sulphate.

Alginic acid is a naturally occurring unbranched binary copolymer of guluronic acid (G) and its C-5 epimer mannuronic acid (M). It has been found that the G- and M-units are joined together in a blockwise fashion. The salts of alginic acids are generally named alginates. Alginates are extracted in large amounts from brown seaweed. The proportions of G and M in the polymer, and the distribution of G and M blocks in the polymer, depends on the source of the alginate (Cf. Carbohydrates in Europe 1996, 14, 6-31).

In most applications alginate gel formation is achieved with calcium ions. However, alginate form gels with most di- and multivalent cations. Monovalent cations and Mg^{2+} ions do not induce gelation while ions like Ba^{2+} and Sr^{2+} will produce stronger alginate gels than Ca^{2+} . The gel strength is dependent upon the guluronic acid content and also on the average number of G-units in the G-blocks.

Crosslinked alginates are used, for example, as rheology control additives, as wound dressings or for immobilizing materials such as plant cells, mammalian cells, yeasts, bacteria, vaccines or food products. Alginate gel formation is achieved with calcium ions in most applications.

A number of different methods for the immobilization of biomaterials in alginate beads have been developed. A commonly used way to form alginate gel beads is by adding an alginate solution dropwise to a solution of gelling ions, for example calcium chloride. The droplet size will determine the size of the spheres. A syringe needle has been used for the formation of alginate droplets. However, reduction in bead size is limited by the syringe needle diameter and the viscosity of the solution. As a result, beads with a diameter of less than 1 mm are difficult to produce. Reduction in bead size has been attempted by air jets impinging on the needle (Miyawaki et al., Agric. Biol. Chem. 1980, 44, 2865), electrostatic pulses (EP 0 167 690 B1) or vibrating needles (Hulst et al., J. Chem. Technol. Biotechnol. 1985, 35B, 198).

There is a demand for microspheres with a mean diameter of about 10 μm , since stable non-sedimenting suspensions may be prepared comprising microspheres of this size, and because microspheres having a diameter of about 10 μm may be taken up by cells allowing a more efficient drug release inside cells (D. T. O'Hagan, J. Anat. 189, 1996, 477-482).

Fine droplets of an alginate solution may be generated by using a spray head, as disclosed, for example, in US 5,387,522 and US 6,465,226. Alginate particles having a diameter of about 200 μm to about 300 μm have been obtained by the above method.

Ca-alginate microspheres may also be obtained by using emulsification methods. Poncelet, et al., Appl. Microbiol. Biotechnol. 1995, 43, 644 have described the production of alginate microspheres by emulsification/internal gelation of alginate sol dispersed within vegetable oil. Gelation was initiated within the alginate sol by reduction in pH releasing calcium from an insoluble complex. Alginate microspheres with mean diameters ranging from 50 μm to 1000 μm were obtained.

Even finer alginate microspheres having a diameter of about 10 μm have been obtained by further optimizing the effects of various operational and formulation factors in the emulsification technique (D. Lemoine, et al. International Journal of Pharmaceutics 1998, 176, 9).

Emulsification techniques for the generation of alginate microspheres are using oils and/or organic solvents. Sensitive biomolecules (proteins, enzymes) may be incompatible with oils

and/or organic solvents. Removal of oils and/or organic solvents from alginate microspheres is a tedious and incomplete process.

Therefore, there is a need for a process for the manufacture of microspheres made from a crosslinkable anionic polysaccharide having a size of about 3 - 20 μm and being devoid of traces of oils and/or organic solvents.

Surprisingly, it has been found that microspheres of crosslinked anionic polysaccharides having a diameter of about 3 to 20 μm which are completely free of oils and/or organic solvents may be obtained by generating fine liquid aerosol droplets from a solution of a water soluble anionic polysaccharide into a stream of a gas and subsequently introducing the stream of gas comprising these droplets into a gelling solution comprising gel-forming cations.

Therefore, in one aspect, the invention relates to a process for preparing microspheres comprising a crosslinked anionic polysaccharide, the process comprising:

- (a) producing liquid aerosol droplets (13) from a solution comprising an ionically crosslinkable anionic polysaccharide into a continuous gas stream by using an ultrasonic nebulizer;
- (b) transferring the gas stream into a gelling solution (10) comprising di- and/or multivalent metal cations, whereby crosslinked anionic polysaccharide microspheres (14) are formed,
- (c) separating the polysaccharide microspheres, and
- (d) optionally, filtering the polysaccharide microspheres through a screen.

In another aspect, the invention relates to a system for preparing microspheres comprising a crosslinked anionic polysaccharide, the system comprising

- (a) an ultra sound generator (1) situated in a nebulizing chamber (2) which is filled with a solution comprising an ionically crosslinkable anionic polysaccharide;
- (b) a radiator coil (4) attached to the nebulizing chamber;
- (c) optionally, means (6) for keeping the gas-fluid level (5) in the nebulizing chamber (2) at a predetermined constant level;
- (d) a gas inlet (7) attached to the nebulizing chamber (2)
- (e) a vessel for the gelling solution (9), equipped with agitation means (11); and

(f) a transfer tubing (8) attached to the nebulizing chamber, connecting nebulizing chamber and vessel, wherein the tubing is adapted to submerge into the gelling solution (10).

Short description of the drawings:

Fig. 1 is a schematic picture of the system for the production of microspheres according to the invention.

Fig. 2 is a size distribution of alginate microspheres manufactured by the process of the invention.

Fig. 3 is a picture of alginate microspheres manufactured according to the process of the invention wherein the diameter of selected microspheres has been determined.

Key part of the system is an ultrasonic nebulizer. A device as being used in air conditioning systems for air moistening may be used. A suitable nebulizer is, for example, the air moistening device SCA 1000, manufactured by Stulz GmbH, D-22457 Hamburg.

Preferably, the radiator coil (4) is connected to means for keeping the temperature of the solution to be nebulized at a predetermined range.

Preferred temperature ranges of the solution to be nebulized are of from 15 to 50 °C, in particular of from 25 to 35 °C.

It is preferred to dip the tubing connecting nebulizing chamber (2) and vessel (9) as deep as possible into the bath of the gelling solution (10) to allow the aerosol droplets (13) comprising anionic polysaccharide to interact with the polyvalent cations of the gelling solution to form crosslinked microspheres (14).

It is further preferred that the part of the tubing which submerges into the gelling solution comprises dispenser holes (12).

The process of the invention may be carried out with different crosslinkable anionic polysaccharides. Preferred crosslinkable anionic polysaccharides are selected from the group consisting of an alginic acid, a carrageenan, a cellulose sulphate, a gellan, a pectin and water soluble salts thereof. Most preferred anionic polysaccharide is alginic acid or a water soluble salt thereof.

Particularly preferred crosslinkable anionic polysaccharides are Na^+ -, K^+ -, NH_4^+ -, and Mg^{2+} -salts of alginic acid and Na^+ -, K^+ -, and NH_4^+ -salts of gellan, carrageenan and cellulose sulfate.

Preferably, the liquid to be nebulized (3) comprises the crosslinkable anionic polysaccharide in a concentration of from 0.1 % to 5.0 % by weight, particularly of from 0.75 % to 1.5 % by weight.

A particularly preferred liquid to be nebulized comprises of from 0.75 % to 1.5 % by weight low viscosity sodium alginate.

The stream of gas may be generated by pressurized air. However, other gases, in particular inert gases, for example nitrogen or argon, are also well suited. The gas should be purified before usage.

The gelling solution comprises a salt of a gel-forming cation in a concentration of from 0.1 % by weight up to saturated solutions. Preferred concentrations of the salt comprising a gel-forming cation are 0.5 to 5 % by weight. Gel-forming cations are, for example, Pb^{2+} , Be^{2+} , Ca^{2+} , Ba^{2+} , Sr^{2+} , Zn^{2+} , Cu^{2+} , Mn^{2+} , Co^{2+} , Fe^{2+} and Fe^{3+} . A particularly preferred salt comprising a gel-forming cation is CaCl_2 .

It is preferred that the gelling solution is a solution essentially consisting of water, an inorganic salt comprising one or more gel-forming cations selected from the group consisting of Ba^{2+} , Sr^{2+} , and Ca^{2+} and of from 0.05 to 0.15 % by weight of a surfactant.

It is understood that the gelling solution has to be adapted according to the gelling characteristics of a specific crosslinkable anionic polysaccharide. A person skilled in the art knows how to select suitable divalent or polyvalent cations.

In the case of the formation of alginate microspheres by the present process, it is preferred to apply a gel-forming cation selected from the group consisting of Ba^{2+} , Sr^{2+} , and Ca^{2+} . Most preferred cation for crosslinking alginate is Ca^{2+} .

The gelling solution is preferably essentially aqueous, but may comprise up to 25 % by weight, preferably 0 to 10 % by weight of one or more cosolvents. Suitable cosolvents

include alcohols, for example, ethanol, isopropanol, glycols, and glycerin; esters, for example, ethyl acetate; or amides, for example, dimethyl formamide.

Preferably, the gelling solution comprises up to 1.0 % by weight, in particular of from 0.05 to 0.15 % by weight of a surfactant. Suitable surfactants are, for example, polyoxyethylene-sorbitans (e.g., TWEEN®), polyoxyethylated glycol monoethers, or surfactants comprising a block copolymer of ethylene oxide and/or propylene oxide (e.g. poloxamers or poloxamines). A particularly preferred surfactant is poly(oxyethylene)20-sorbitane monolaureate (TWEEN® 20).

The alginate microspheres prepared by the method described in the invention are the first microspheres in the low micrometer range which are produced without using an emulsion method. Thus, no oil or organic solvent is needed which might interfere with biomolecules or living cells. Therefore, no subsequent tedious purification steps are required to remove any residual oil or organic solvent.

Small alginate microspheres can be added to solutions (e.g. juice, medicinal drops) without sedimentation. Thus, a homogenous suspension of a drug entrapped in an alginate microsphere can be prepared.

Additionally, as mentioned above, microspheres with a mean diameter below 10 μm can be taken up by a cell which allows a more efficient drug release inside cells.

Furthermore, the size distribution of the microspheres produced by the present process is very narrow and reproducible (generally about 2 to 15 μm , with an average diameter of about 8 μm) compared to other methods described in literature. Preferably, ≥ 95 % of the microspheres produced by the present process have a diameter of from 3 to 20 μm . This narrow size distribution of the alginate microspheres guarantee a more homogeneous loading and release of drugs or biomolecules.

For a controlled drug release it is very important to have a constant release rate to avoid over or under dosing. To achieve this, a tight control of the surface to volume ratio of the microspheres is necessary. The surface to volume ratio is determined by the size distribution. Thus, a narrow size distribution results in a reproducible surface to volume ratio and finally a controlled drug release.

Example: Preparation of Alginate Microspheres

A 1% wt. solution of sodium alginate (Sigma, from brown algae *Macrocystis pyrifera* (kelp), low viscosity) in ultra pure water is filled into the nebulizing chamber (2) of a system according to Fig. 1. The temperature of the radiator coil (4) is adjusted to maintain a temperature of from 25 to 30 °C in the nebulizing chamber. The transfer tubing (8) is dipped into an ultra sound bath (35 kHz) which is filled with 1500 ml of a solution gelling solution of 5 % by weight of CaCl_2 and 0.1 % by weight of TWEEN 20 (Poly(oxyethylene)20-sorbitane monolaureate) in water. The pressured air is adjusted to produce a slight stream of air bubbles through the CaCl_2 bath. Then, the ultra sound generator is turned on for 30 min. The precipitation bath turns turbid which indicates the formation of alginate spheres. To remove very large particles which are generated by condensation of aerosol droplets on the walls of the transfer tube, the alginate - CaCl_2 mixture is filtered through a 50 μm screen cloth. The alginate microspheres are separated from the CaCl_2 bath by centrifugation (10 min, 1000 x g) and decanting of the supernatant. The size of the alginate microspheres was determined to be between 5 and 10 μm by microscopy imaging (see Fig. 3).

The size distribution of the alginate microspheres is determined with a laser scattering particle size distribution analyzer (LA-910 from Horiba, Ltd. Kyoto, Japan). A refractive index of 1.35 is used for the alginate microspheres. More than 90% of the spheres are in a range of 5 to 13 μm with the average at 8 μm (Fig. 2).

Claims:

1. A process for preparing microspheres comprising a crosslinked anionic polysaccharide, the process comprising:
 - (a) producing liquid aerosol droplets (13) from a solution comprising an ionically crosslinkable anionic polysaccharide into a continuous gas stream by using an ultrasonic nebulizer;
 - (b) transferring the gas stream into a gelling solution (10) comprising di- and/or multivalent metal cations, whereby crosslinked anionic polysaccharide microspheres (14) are formed,
 - (c) separating the polysaccharide microspheres from the gelling solution, and
 - (d) optionally, filtering the polysaccharide microspheres through a screen.
2. The process according to claim 1, wherein the anionic polysaccharide is selected from the group consisting of an alginic acid, a carrageenan, a cellulose sulphate, a gellan, a pectin and water soluble salts thereof.
3. The process according to claim 1, wherein the anionic polysaccharide is an alginic acid or a water soluble salt thereof.
4. The process according to any one of claims 1 to 3, wherein the metal cation of the gelling solution is selected from the group consisting of Pb^{2+} , Cu^{2+} , Ba^{2+} , Sr^{2+} , Cd^{2+} , Ca^{2+} , Zn^{2+} , Co^{2+} , and Ni^{2+} .
5. The process according to any one of claims 1 to 3, wherein the metal cation of the gelling solution is selected from the group consisting of Ba^{2+} , Sr^{2+} , and Ca^{2+} .
6. The process according to any one of claims 1 to 3, wherein the metal cation of the gelling solution is Ca^{2+} .
7. The process according to any one of claims 1 to 6, wherein the gelling solution additionally comprises up to 1 % by weight of a surfactant.
8. The process according to claim 7, wherein the surfactant is present in an amount of from 0.02 to 1.0 % by weight, preferably of from 0.05 to 0.15 % by weight.

9. The process according to claim 7 or 8, wherein the surfactant is selected from the group consisting of polyoxyethylene-sorbitans and surfactants comprising a block copolymer of ethylene oxide and/or propylene oxide.

10. The process according to any one of the claims 1 to 9, wherein the temperature of the anionic polysaccharide solution according to step (a) is kept within a temperature of from 15 to 50 °C, preferably within 25 to 35 °C.

11. The process according to claim 1, wherein the anionic polysaccharide is an alginic acid or a water soluble salt thereof, the metal cation is Ca^{2+} ; and wherein the gelling solution comprises of from 0.05 % to 0.15 of poly(oxyethylene)20-sorbitane monolaureate.

12. A system for preparing microspheres comprising a crosslinked anionic polysaccharide, the system comprising

- (a) an ultra sound generator (1) situated in a nebulizing chamber (2) which is filled with a solution comprising an ionically crosslinkable anionic polysaccharide;
- (b) a radiator coil (4) attached to the nebulizing chamber;
- (c) optionally, means (6) for keeping the gas-fluid level (5) in the nebulizing chamber (2) at a predetermined constant level;
- (d) a gas inlet (7) attached to the nebulizing chamber (2)
- (e) a vessel for the gelling solution (9), equipped with agitation means (11); and
- (f) a transfer tubing (8) attached to the nebulizing chamber, connecting nebulizing chamber and vessel, wherein the tubing is adapted to submerge into the gelling solution (10).

13. Microspheres of a crosslinked anionic polysaccharide, wherein ≥ 95 % of the microspheres of have a diameter of from 3 to 20 μ and wherein the microspheres are essentially free of oil and organic solvents.

14. Microspheres according to claim 13, which comprise a crosslinked Ca^{2+} -alginate.

Abstract

The invention relates to a process for preparing microspheres of a crosslinked anionic polysaccharide comprising:

- (a) producing liquid aerosol droplets (13) from a solution comprising a water-soluble anionic polysaccharide into a continuous gas stream by using an ultrasonic nebulizer;
- (b) transferring the gas stream into a gelling solution (10) comprising di- and/or multivalent metal cations, whereby crosslinked anionic polysaccharide microspheres (14) are formed,
- (c) separating the polysaccharide microspheres, and
- (d) optionally, filtering the polysaccharide microspheres through a screen.

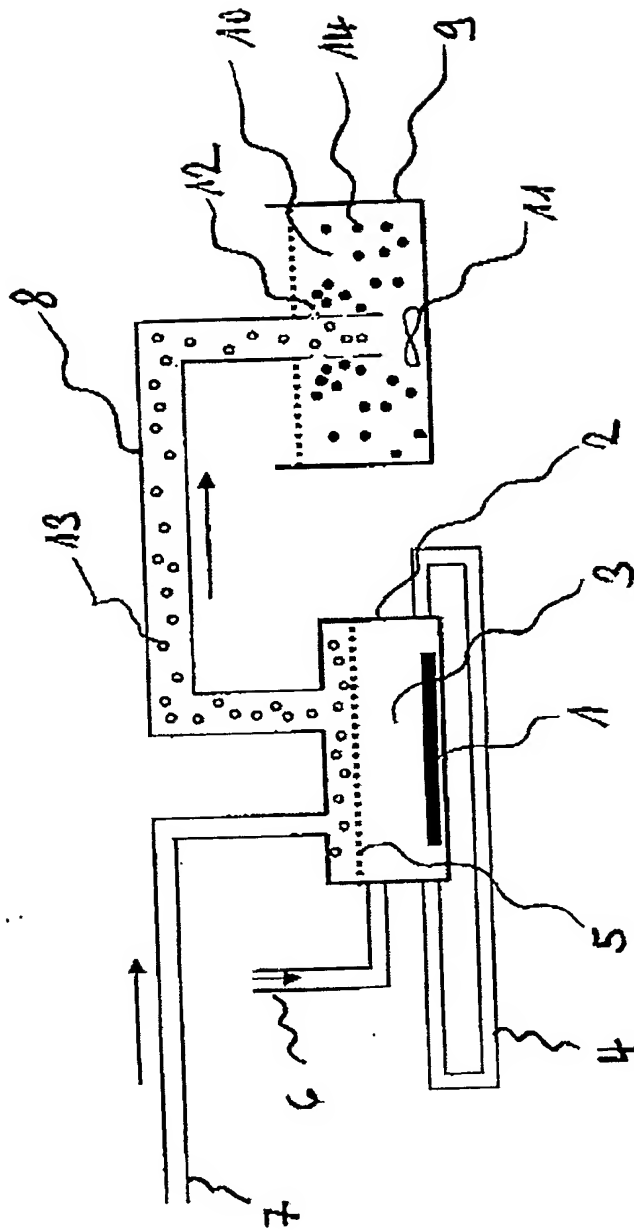


FIG. 1

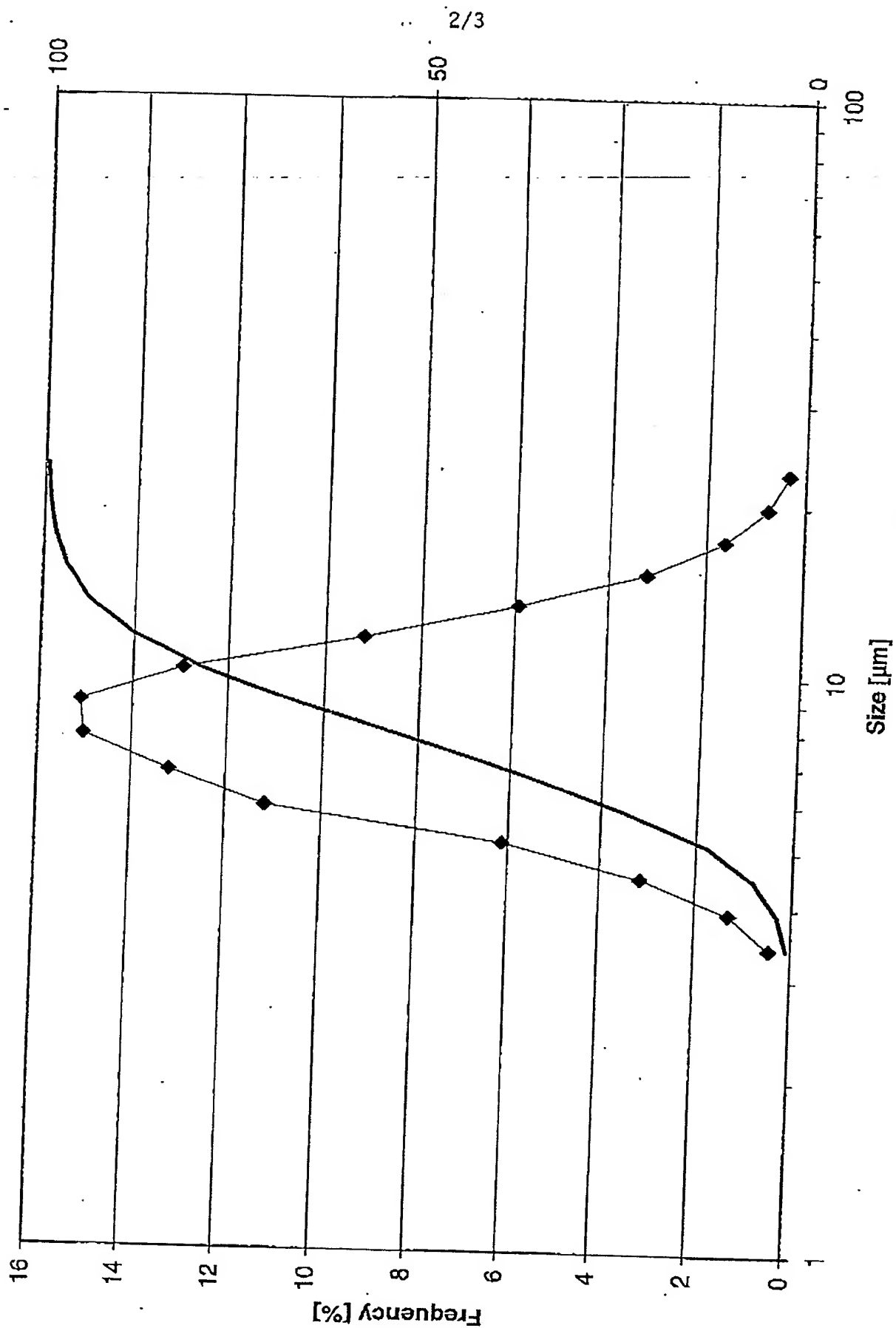


FIG. 2

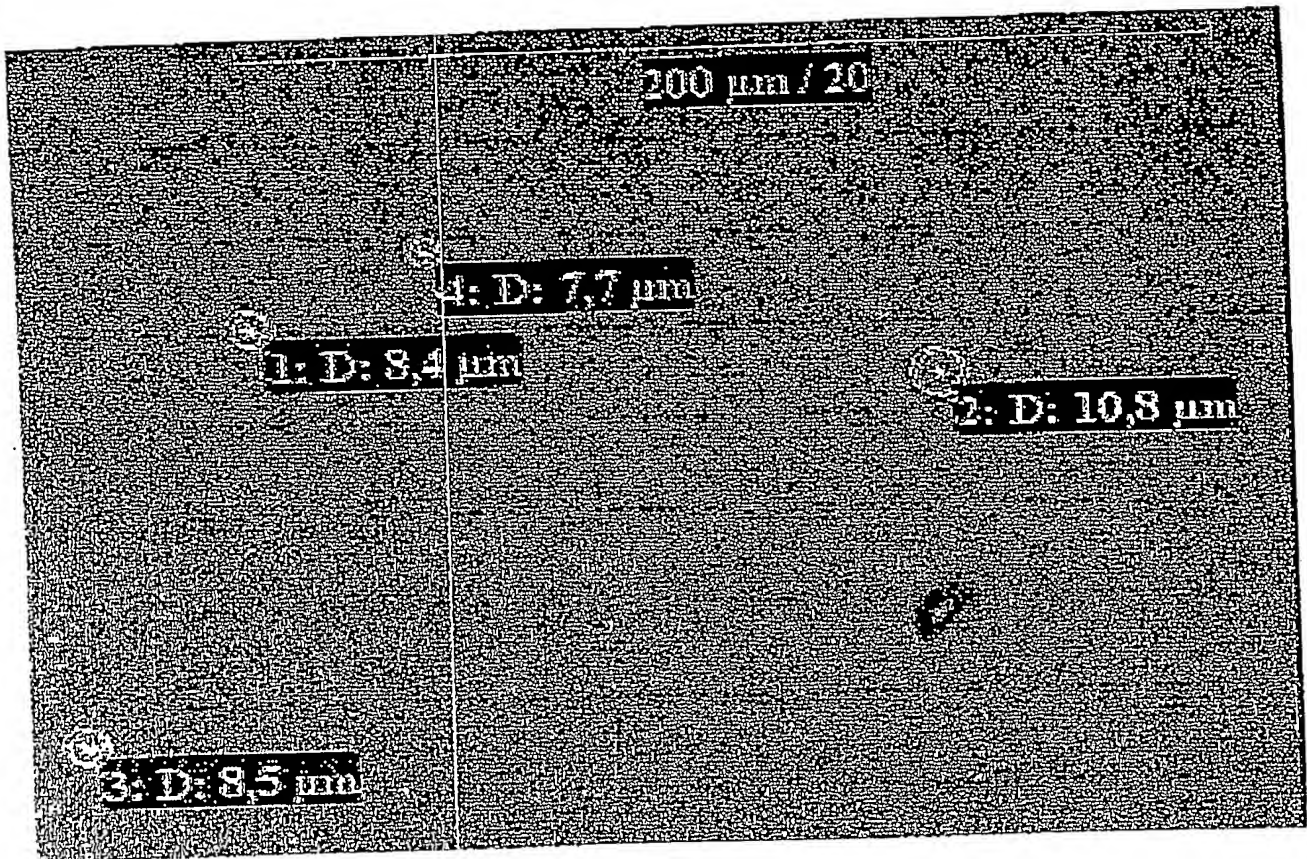


FIG. 3

